WO 2005/044838 PCT/US2004/034679

- 38 -

CLAIMS

We Claim:

1. A method for determining a T-cell epitope of a protein, wherein said protein is a bone morphogenetic protein (BMP), comprising the steps of:

- (a) obtaining from a solution of dendritic cells and a solution of naïve CD4+ and/or CD8+ T-cells from a single human blood source;
- (b) differentiating said dendritic cells, in said solution of dendritic cells, to produce a solution of differentiated dendritic cells;
 - (c) preparing a pepset of peptides from said protein;
- (d) combining said solution of differentiated dendritic cells and said naïve CD4+ and/or CD8+ T-cells with said pepset, wherein said pepset comprises said T-cell epitope; and
 - (e) measuring the proliferation of said T-cells in said step (d).
- 2. The method of Claim 1, wherein said protein is selected from the group consisting of BMP-7 and BMP-14.
- 3. The method of Claim 1, wherein said pepset comprises a peptide having the sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5.
- 4 The method of Claim 1, wherein said pepset comprises a peptide having the sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8.
- 5. The method of Claim 1, further comprising the step of modifying said protein to produce a variant protein, wherein said variant protein exhibits an altered immunogenic response as compared to said protein.
- 6. A peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8.

- A method of reducing the immunogenicity of a protein, wherein said protein is a 7. bone morphogenetic protein, comprising the steps of:
 - (a) identifying at least one T-cell epitope in said protein by
 - contacting an adherent monocyte-derived dendritic cell that has been differentiated by exposure to at least one cytokine in vitro, with at least one peptide comprising said T-cell epitope; and
 - contacting said dendritic cell and said peptide with a naïve T-cell, (ii) wherein said naïve T-cell has been obtained from the same source as said adherent monocyte-derived dendritic cell, and whereby said T-cell proliferates in response to said peptide; and
 - modifying said protein to neutralize said T-cell epitope to produce a (b) variant protein, such that said variant protein induces less than or substantially equal to the baseline proliferation of said naïve T-cells.
- 8. The method of Claim 7, wherein said T-cell epitope is modified by substituting a portion of the amino acid sequence of said T-cell epitope with an analogous sequence from a homolog of said protein.
- 9. The method of Claim 7, wherein said T-cell epitope is modified by substituting the amino acid sequence of said T-cell epitope with a sequence which substantially mimics the major tertiary structure attributes of said T-cell epitope.
- 10. The method of Claim 7, wherein said protein is selected from the group consisting of BMP-7 and BMP-14.
- 11. The method of Claim 7, wherein said epitope region comprises an amino acid sequence, wherein said amino acid sequence is selected from the group consisting of SEO ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7 and SEQ ID NO:8.
- 12. A method for producing a variant protein having reduced allergenicity comprising the steps of:
 - obtaining a naturally-occurring protein, wherein said naturally-occurring a) protein is a bone morphogenetic protein, and preparing fragments of said naturally-

occurring protein;

- b) contacting said fragments of said naturally-occurring protein with a first solution comprising naïve human CD4+ or CD8+ T-cells and differentiated dendritic cells;
- c) identifying an epitope region of said naturally-occurring protein, wherein said identifying comprises measuring the ability of said fragments of said naturally-occurring protein epitope region to stimulate proliferation of said naïve human CD4+ or CD8+ T-cells; and
- d) modifying at least one amino acid in said epitope region identified in step c), to produce said variant protein.
- 13. The method of Claim 12, further comprising the step of comparing the ability of said fragments of said naturally-occurring protein to stimulate proliferation of said naïve human CD4+ or CD8+ T-cells with the ability of said fragments of said variant protein to stimulate proliferation of said naïve human CD4+ or CD8+ T-cells.
 - 14. The method of Claim 12, wherein said protein is a bone morphogenetic protein.
- 15. The method of Claim 14, wherein said bone morphogenetic protein is selected from the group consisting of BMP-7 and BMP-14.
- 16. The method of Claim 12, wherein said epitope region comprises an amino acid sequence, wherein said amino acid sequence is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7 and SEQ ID NO:8.